Detection of DNA with Quant-iT™ PicoGreen® dsDNA Reagent in microplate format

The Quant-iT™ PicoGreen® dsDNA Quantitation Reagent from Invitrogen Molecular Probes is an ultrasensitive fluorescent stain used for the quantitation of double-stranded DNA in a solution. In many molecular biology applications the detection and quantitation of small amounts of dsDNA is very important. In combination with Thermo Scientific Fluoroskan Ascent or Fluoroskan Ascent FL, this reagent provides rapid and easy determination of dsDNA, offering ultimate sensitivity and a wide dynamic range.

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Introduction

The PicoGreen reagent is an ultrasensitive fluorometric stain used for the quantitation of dsDNA in a solution. In a wide variety of molecular biology applications the detection and quantitation of small amounts of dsDNA is very important. This reagent is much more sensitive than the most commonly used absorbance measurement at 260 nm (A260). When measuring the absorbance, single-stranded nucleic acids and nucleotides interfere with the obtained signal.

The commonly used bisbenzimide dyes of Hoechst, 33258 and 33342, are affected less by interfering substances than the absorbance measurement but the sensitivity is not as good as with PicoGreen. Only one dilution of the reagent is needed for the whole dynamic range instead of two different fluorochrome dilutions which are generally needed with the Hoechst dye.

In combination with Thermo Scientific Fluoroskan Ascent or Fluoroskan Ascent FL, the PicoGreen reagent provides rapid and easy determination of dsDNA, offering a wide dynamic range.

Reagents and materials

PicoGreen dsDNA Quantitation Kit, Invitrogen Molecular Probes, #P-7589 or equivalent.

The kit contains:

- PicoGreen dsDNA quantitation reagent, 1 ml, stock solution in DMSO
- 20 x TE buffer, 25 ml, 200 mM Tris-HCl, 20 mM EDTA, pH 7.5
- Lambda DNA standard, 1 ml, 100 µg/ml in TE buffer

Instruments

Thermo Scientific Fluoroskan Ascent or Fluoroskan Ascent

FL microplate reader equipped with fluorescein filters (Ex 485 nm, #1424852, and Em 538 nm, #1425382).

Other materials needed

- White 96-well plates (Thermo Scientific Microlite1+, #7571) or white 384-well plates (Thermo Scientific Microlite1, #8155)
- Finnpipettes and Finntips for preparing and dispensing samples
- Sterile plastic tubes for making dilutions
- Sterile, distilled DNase-free water

Preparation of reagents

1 x TE buffer

Prepare a 1 x TE working solution by diluting the concentrate 1:20 with sterile, distilled DNase-free water. Use this TE buffer as a blank and for preparing all the DNA standard dilutions.

PicoGreen working solution

Dilute the PicoGreen reagent 1:200 with TE buffer in a plastic tube. Protect the working solution from light and use it within a few hours after preparation. 100 µl/well of diluted reagent is needed for 96-well plates, and 25 µl/well for 384-well plates.

DNA standard curves

Dilute a small aliquot of the provided Lambda DNA standard 1:50 with TE buffer to prepare a 2 µg/ml DNA stock solution in TE buffer. An aliquot of 100 µl/well of each DNA standard is required for the assay with 96-well plates and 20 µl/well with 384-well plates. Depending on the estimated DNA concentration in the samples, prepare either a high- or a low-concentration range calibration curve with the 2 µg/ml DNA stock solution.

For the standard curve, prepare further dilutions of the 2 µg/ml DNA stock solution in TE buffer, which correspond to 1 000 ng/ml in the final assay volume (both with 96 and 384-well plates). Make additional 1:5 or 1:10 dilutions (= 200 ng/ml, 20 ng/ml and 2 ng/ml) with the TE buffer. Use the TE buffer as a blank.

NOTE! For more information about the procedure, preparation and storage of the reagents, refer to the Invitrogen Molecular Probes Product Information Sheet supplied with the kit.

Procedure

Procedure for 96-well plate assay

Pipette 100 µl of blank, DNA standard dilutions and samples into the wells of a white 96-well plate with a defined number of replicates (having at least 2-3 replicates is recommended). Add 100 µl of the PicoGreen working solution into each well. Mix well and incubate at room temperature for 2 to 5 min protected from light.

Measure the fluorescence with the Fluoroskan Ascent or Fluoroskan Ascent FL fluorometer. Use filters with a 485 nm excitation and a 538 nm emission wavelength.

Procedure for 384-well plate assay

Pipette 25 µl of blank, DNA standard dilutions and samples into the wells of a white 384-well plate with a defined number of replicates (having at least 2-3 replicates is recommended). Add 25 µl of the PicoGreen working solution into each well. Mix well and incubate at room temperature for 2 to 5 min protected from light.

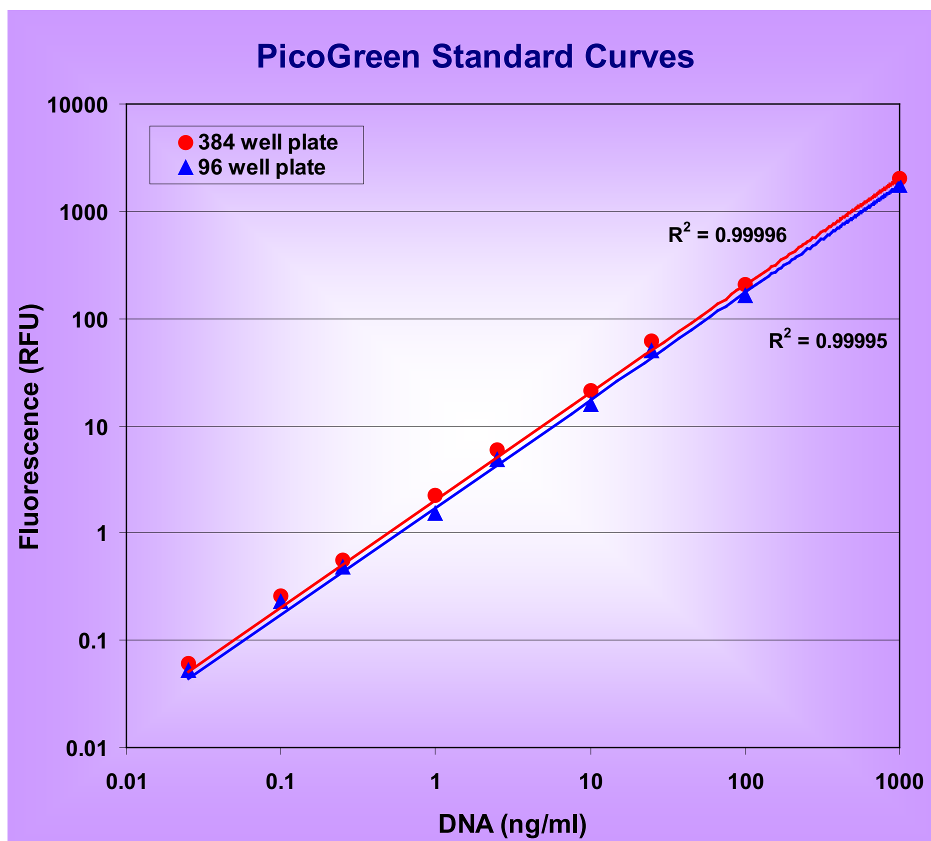
Measure the fluorescence with the Fluoroskan Ascent or Fluoroskan Ascent FL fluorometer. Use filters with a 485 nm excitation and a 538 nm emission wavelength.

Fluoroskan Ascent or Fluoroskan Ascent FL procedure parameters

- In Ascent Software select the area to be measured under General parameters.
- Define the Layout (name the Blanks, Calibrators and Samples).
- Select a Shake step and set the corresponding parameters (total time 2 to 5 min, on time 10 s; this will perform both shaking and incubation).
- Select a Measure step and select the 485/538 filter pair.
- Select single measurement and use normal beam for the 96-well plate and small beam for the 384-well plate, and select an integration time of 20 ms.
- Start the measurement procedure.
- After the measurement has been completed, subtract the average blank value from the standards and samples.
- Generate the standard curve. Select Curve Fit in the Process menu to generate the standard curve and to read the unknown samples.

Results and discussion

Figure 1. Detection of dsDNA with the PicoGreen reagent and Fluoroskan Ascent using the filter combination Ex 485/Em 538. The assay was performed using either white 96-well strip plates (total volume 200 µl/well) or white 384-well plates (total volume 50 µl/well). The PicoGreen assay combined with the Fluoroskan Ascent or Fluoroskan Ascent FL showed perfect linearity over a wide concentration range, resulting in reliable determination of DNA concentration in both low- and high-concentration samples. Calculated detection limits (based on the IUPAC standard 3*SD method) for the assay are 0.02 ng/ml with a 96-well plate and 0.07 ng/ml with a 384-well plate. These results show that this assay combination is by far the most sensitive that is available at the moment.



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References

Web address	Reference	Author	Title
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